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Citrus peel essential oils: a review on composition and antimicrobial activities

Hafizu Ibrahim Kademi[1], Umar Garba[2]
1Department of Food Engineering, Near East University, Cyprus.
2Depatment of Food Science and Technology, Faculty of Agriculture, Bayero University Kano 3011, Nigeria.
hafeezkademi@gmail.com, ugarba.fst@buk.edu.ng, umargarbafst@yahoo.com

ABSTRACT
This review examined the composition and antimicrobial activities of citrus peel essential oils. Citrus peels are the waste product of fruit processing industry rich in essential oil (EO) with many phytochemical compounds of excellent antimicrobial properties against many bacteria and fungi. The citrus peel EOs has been widely used in pharmaceuticals, foods and other industries as preservatives, and its generally regarded as safe (GRAS). The extract can be obtained through extraction different techniques such steam and hydro-distillation. The components of the EOs are important and their quality depends on the qualitative and quantitative characteristic of the oils. Equipment such as gas chromatography (GC)-flame ionization detector (FID) and GC-MS are widely used for their analysis. The growing concern on the serious microbial spoilage and the interest in shifting from synthetic to natural antimicrobial agents leads to research and screening of plant and other vegetable sources to identify new compounds for the manufacturing and industrial uses. Although the antimicrobial properties of EOs have been reviewed extensively, the antimicrobial properties of citrus peels oil have not been extensively discussed. The hydro-distillation methods appeared to be more widely used by many researchers, perhaps due to its efficiency, inexpensive and yield of excellent quality EOs.

Key words: citrus peel, essential oils, antimicrobial properties, chemical composition

INTRODUCTION

The citrus plant belongs to the family *Rutaceae*, comprising of about 17 species found throughout the tropical, subtropical and temperate regions [1; 2; 3]. Among the species, *Citrus indica, Citrus latipes*, *Citrus aurantium*, *Citrus macroptera*, *Citrus megaloxycarpa*, *Citrus jambhiri*, C*itrus ichangenesis* and *Citrus reticulate* are the most common. The genus citrus includes different important fruits such as orange, mandarins, limes, lemons, and citrons grapefruits [4; 5]. Although, there are many groups of plants that are very essential in phytochemistry, citrus plantation has been considered the most valuable for industrial and commercial agricultural practices in the world [6]. The record of citrus cultivation extending back to 2100 BC confirmed that citrus is an ancient crop [7].

In 2012, the world production of citrus was estimated to about 131 million metric tons [8], and it's grown in over 140 countries around the world, with China, Brazil, U.S.A, India, Mexico and Spain as the world leading producers. The citrus peel which is almost one half of the total fruit mass is a rich source of phenolic compounds such as flavanones, flavonols and many polymethoxylated flavones, and are very scarce in other plants. In addition to these compounds, many hydroxycinnamates also exist [9]; [10]. Empirically, hesperidine, naringin, narirutin and eriocitrin are the most popular flavonoids present in the citrus peels [11; 12]. These compounds, plays some excellent roles in physiological, ecological, as well as in foods and pharmaceuticals applications [6; 13; 14]. Historically, citrus peels were found useful in traditional medicinal herbs in some countries such as China, Korea and Japan. It is believed to have cured skin inflammation, indigestion, cough, muscle pain, ringworm infections and reduce blood pressure [5]. The essential oils of this fruits are present in great quantity in the flavado portion [15], the layer consisting of the epidermis covering the exocarp of irregular parenchymous cells, which are completely enclosing numerous glands or oil sacs [4]. The growing interest of shifting from the synthetic antimicrobial agents to natural ones has encourage research on screening of plant material and other vegetable sources to identify new compounds [16]. These essential oils have important application as a natural antibacterial gent for food industry, particularly pasta manufacturing industry which have serious spoilage challenge because of lactic acid bacteria activity. And because of these nutraceutical and economic importance of essential oils of citrus fruits, numerous research has been conducted aiming to identifying their chemical composition and antimicrobial activities [17]. The aim of this work is to review some of the available methods used for extraction of citrus peel essential oils and their compositions as well as the antimicrobial properties of EOs.

CITRUS PEEL OIL

Essential oils are natural extract, volatile and complex compounds characterized by highly stimulating odor and are produced by aromatic

plants as secondary metabolites which have been extracted and concentrated from different part of plants [18; 19]. They are rich sources of flavanoids, alkaloids, coumarins, limonoids, carotenoids, phenolic acid, and many polymethoxylated flavones, which are not mostly found in other plants [5, 20]. They are mostly obtained by steam or hydro-distillation and are known for their antiseptic (i.e. bactericidal, fungicidal, and virucidal) and medicinal properties and their fragrance since middle ages. They are used in food preservation, antimicrobial, perfumes, analgesic, sedative, anti-inflammatory, spasmolytic and medicinal uses [18; 21].

1. EXTRACTION OF ESSENTIAL OILS

This review highlighted some of the widely used techniques for the extraction of citrus peel essential oils. Steam distillation is the most frequently used method to extract essential oils using Clevenger-type apparatus. Other methods of extraction are solvent extraction, simultaneous distillation extraction, supercritical carbon dioxide and microwave ovens. The extract may differ in quality, quantity and composition depending on the climate, soil composition, plant organ, age and vegetative cycle stage [22]. Some of these methods are discussed under the headings below based on the nature of the process.

1.1 Steam distillation (SD)

SD is another type of distillation or separation process for a heat sensitive oils. It is one of the officially approved ancient methods for the extraction of EOs from plant sources [23]. It is economical and faster than many other techniques [24]. The extraction of citrus peel essential oil by SD from citrus fruit peels such as lemon, mandarin and orange peels were reported by many researchers [24; 25; 26; 27; 28; 29]. It has also been employed for spices like cinnamon [30]. The process description involves feeding the plant materials into the chamber without maceration in water [23], and then passing a steam (1 kg/h; 96-100°C) at 1.1 atm for about 3 h through a 5 L round-bottomed flask containing 1.0-1.5 kg of chopped lemon, mandarin or orange fruit peel. The condensed volatile oils were decanted from brine and dried over anhydrous Na_2SO_4 [25]. Sikdar et al. [29] stated that an optimum amount of citrus oil about 2.4 mL/100g of orange peel can be extracted by SD at optimum temperature of 96°C for 60 minutes using a solid to solvent ratio of 100g/200mL. However, higher yield of oil can be obtained by improved SD example 4.24% for *C. sinensis* and 5.87% for *C. reticulate* as compared to conventional steam distillation with 2.48% and 0.98% respectively [24].

1.2 Hydro-distillation (HD)

This method has been reported by many researchers [31; 32; 33; 34; 35; 36]. Extraction of citrus essential oil by HD involve 3-4 h extraction in water to obtain a mixture of citrus oil and water which can later be settled into two liquid layers and then separated. Hydro-distilled pure oil obtained can be stored in deep brown sealed vials at 4°C until analysis [32]. In another procedure, the essential oil after extraction is dried over anhydrous sodium sulphate and stored under N_2 in sealed at 4°C [4]. A Clevenger-type apparatus can be used for HD [37]. This method yields volatile EOs consisting of mainly monterpenes and few sesquiterpenes. A typical yield of 1.4% was reported from 150 g of fruit peels [35]. The drying of the peel was also found to increase the oil yield. A yield of oven-dried peels of *C. reticulata, C. sinensis and C. paradisii* ranged from 0.3-0.5, 0.24-1.07 and 0.2-0.4 g/100 g respectively [33]. In another research, a yield of 0.9% and 0.75% were recorded from the peel of *C. medica cv. 'liscia' and C. medica cv. 'rugosa'* [38]. However, losses in volatiles compounds, longer extraction time, thermal and hydrolytic degradation of some components are part of the disadvantages of this method [31].

1.3 Hand pressing/Mechanical pressing

The hand pressing methods is mainly employed for laboratory purpose. It is also called cold pressing or mechanical pressing a. It has been reported by some researchers [20; 17]. The hand pressing method involved manual pressing of the flavado layer to exposed oil sac. The oil can be collected in a brine solution and kept on ice. The extract can then be centrifuged at 6000 rpm for 20 minutes, and dried in anhydrous sodium sulphate. The oil obtained can be stored at -21°C for further analysis of the components [20]. A machine can also be employed to squeeze the citrus peel at room temperature for the release of EOs which are washed in chilly water and further isolate the oil the decantation or centrifugation [23]. However, this method has not been widely used may due to lower yield as compared to the solvent, steam or hydro-distillation processes.

1.4 Solvent extraction

Extraction using ethyl acetate [8]; chloroform [24]. Ethanol and n-Hexane were also reported for the extraction of EO from *Ocimum Basillicum* [27]. Jayaprakasha et al. [39] reported the extraction of EO from *Citrus reticulate* peel using acetone, chloroform and hexane for the antimicrobial test. Solvent extraction has also been used for commercial extraction of EOs using hexane. According to Chanthaphon et al. [4] 500g of citrus peels was

grinded into fine powder, soaked in 2 litres of ethyl acetate and then shake at 130 rpm for 8 h. The filtrate was then dried over anhydrous sodium sulphate. The ethyl acetate was removed by rotary-vacuum evaporation, and removed completely using nitrogen evaporator to obtain a dry ethyl acetate extracts, which was stored in under N_2 in sealed vials at 4°C. The yield was calculated as follows:

$$\text{Yield} = \frac{\text{Weight of extract recovered}}{\text{Weight of fresh citrus peel}} * 100$$

1.5 Microwave-assisted Hydro-distillation

Microwave is a type of electromagnetic wave with frequency range of 300MHz to 300GHz which can produce heat when penetrate and interact with polar molecules like water present in a biomaterial [40]. It is an advance HD method in which the use of microwave is employed to increase the efficiency of the extraction [31; 40; 41]. This method gives a yield higher than obtained by SD because of the synergistic effect of heat and mass transfer [31]. The efficiency of the process depends on the value of the dielectric constant of water and the matrix [41]. This method was adopted to reduce waste, avoid consumption of water and solvent; and allow for saving substantial amount of energy. It also permits for the fast and efficient extraction. Shakir and Salih [40] reported a maximum yield of 1.15%, 1.12% and 0.94% for orange, lemon and mandarin respectively using a power (135W). A yield of value of 5.43 ± 0.03% was obtained when using microwave-assisted steam distillation at 500 W optimized power as compared to 5.45 ± 0.04% for the SD method [41]. Another method using microwave hydro-diffusion and gravity was also reported [43].

2. CHEMICAL COMPOSITION OF CITRUS PEEL ESSENTIAL OILS

The qualitative and quantitative composition of EOs depends on the components present [3], and in turns affect its antimicrobial potential [44]. The citrus essential oil is mostly analysed using different equipment such as gas chromatography (GC)-flame ionization detector (FID) and GC-MS [45]. A Hewlett-Packard gas chromatogram of different models, equipped with a flame ionization detector (FID) supported by electronic integrator were mostly used [26]. However, GC-MS is important equipment which is highly efficient for the analysis of volatile and semi-volatile organic compounds, and separating mixtures into individual constituents with a temperature controlled capillary column. The individual constituents can be analyse using their mass spectra. The GC-MS analyses of citrus peel components are usually carried out on chromatogram equipped with a Hewlett-Parkard MS computerized system of different models. The components identification is based on GC retentions, computer matching with NBS library [26].

The composition of the essential oil varies depending on the ripeness of fruits, storage condition and extraction method [46]. It contains 85-99% volatile and 1-15% non-volatile components [3; 28]. Almost 200 different chemicals compounds were described from orange oil by 1970, of which 100 had been identified [46]. About 37 major components were identified from *Citrus reticulata* [46], and the major components were limonene (46.7%), geranial (19.0%), neral (14.5%), geranyl acetate (3.9%), geraniol (3.5%), β-caryophyllene (2.6), nerol (2.3), citronellal (1.3%), and neryl acetate (1.1%). Citrus oil also composed of about 97% monoterpenes with alcohols, aldehydes and esters being the lowest percentage components, with values ranging between 1.8 to 2.2% [48]. Kamal, *et al.* [33]; could identify about 16-27, 17-24 and 18-40 chemical constituents in the peel essential oil of *C. reticulate, C. sinensis* and *C. paradisii* respectively, and the content of limonin was 64.1-71.1% *(C. reticulata)*, 66.8-80.9% *(C. sinensis)* and 50.8-65.5% *(C. paradisii)*. Twenty-one components were also identified in the peel of Hatian Pummelo (*Citrus Drandis Osbeck)* and monoterpenes and sesquiterpene hydrocarbons were the principle components (96.64% w/w of the total oil). The limonene was observed to be the dominants (89.96±1.64%) as reported [49].

Chemical analysis of *Citrus pseudolimon* and *Citrus grandis* peel EO also revealed the presence of about thirty-six and thirty-three total components, with limonene content of 47.07% and 71.48% respectively [28]. The phytochemical screening of methanolic extracts (peels, leaves and flowers) and unripe epicarp of orange fruit (*Citrus sinensis*) have also revealed the presence of tannins, flavonoids, polyterpenes, alkaloids, saponin, terpenoids, and amino acids [50].

3. ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL

The antimicrobial activities essential oil of citrus among others, has been of interest to foods, pharmaceuticals and cosmetic industries [20]. Many essential oils were used in preparation of skin care and for acne control [51]. The antimicrobial activity can be testes using well-diffusion method and disc diffusion methods [50; 52]. Citrus peels, especiallyy of lemon peels possess a strong antimicrobial

property against some medically important microbes such as *Pseudomonas aeruginosa, Salmonella typhi* and *Micrococcus aureus* [53]. The disc diffusion method and minimal inhibitory concentration determination revealed the antimicrobial activity of the essential oil from the peel of Htian Pummelo (*Citrus Drandis Osbeck*) against *Penicillium chrysogenum ATCC 10106, Bacillus subtilis ATCC 21616, Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922* [49]. The EOs of *C. pseudolimon* and *C. grandis* also shows strong and moderate activity respectively against different bacterial and fungus strains [28]. Kirbaşla et al. [20] reported the antimicrobial activities of Turkish *Citrus* peel oils. The peel oils of lemon (*Citrus limon (L.*) *Burm. f.*), grapefruit (*Citrus paradisi Macfayden*), bergamot (*Citrus bergamia Risso et Poit.*), bitter orange (*Citrus aurantium L.*), sweet orange (*Citrus sinensis (L.) Osbeck*), and mandarin (*Citrus reticulata Blanco*) were evaluated using disk diffusion method towards 9 bacteria and the results was compared with those for penicillin-g, ampicillin, cefotaxime, vancomycine, oflaxacin and tetracycline. Antifungal activities were reported for *Kluyveromyces fragilis*, *Rhodotorula rubra*, *Candida albicans*, *Hanseniaspora guilliermondii* and *Debaryomyces hansenii* yeasts, and the results were referenced against nystatin, ketaconazole and clotrimazole antifungal agents. The Citrus peel oils showed strong antimicrobial activity against the test organisms. Lemon and bergamot peel oils have a little higher activity than the other Citrus peel oils. The screening of oil peel of orange (*Citrus sinensis*) against some medically important microorganism; Gram-positive bacteria *(Staphyloccocus aureus 001, S. aureus ATCC 25923, Enteroccocus feacalis 002, E. feacalis ATCC 295212);* Gram-negative bacteria *(Pseudomonas aeruginosa 003, Escherichia. Coli 004, E. coli ATCC 29522)* and fungi *(Candida albicans 010, C. albicans ATCC 90028)* was conducted. And minimum inhibitory concentration of 1.65-422 mg/mL was estimated using disc diffusion assay. The Orange peels oil was found to exhibit an inhibitory effect against all the microorganism [19].

EL-Hawary et al. [7] also investigated the antiviral activity of essential oils of four cultivars of *Citrus deliciosa var. Tangarina* was tested by hemagglutination test of washed chicken erythrocytes. Positive test was obtained when different citrus essential oils were used to inoculate the allantoic amniotic fluid of embryonatic eggs, suspension of H5N1 virus and antibiotic indicates inactivated H5N1 virus. The effectiveness of Essential oil from Grapefruit (*Citrus paradisi*) to inhibit wild food-borne spoilage and pathogenic bacteria strain was determined. The *Citrus paradisi* essential oil inhibited growth of *Escherichia coli*, *Staphylococcus aureus, Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. diacetylactis, Leuconostoc mesenteroides subsp. dextranicum and Lactobacillus plantarum.* The lowest concentration of essential oil (4.29 ppm) is required to *inhibit Lactococcus lactis subsp. lactis* 207c VCOR (8.27±0.13 log10 CFU/ml) according to the Minimum Inhibitory Concentration [17]. Ngele et al. [52] investigated the antibacterial activity of extract of unripe epicarp of *Citrus sinensis* against *Escherichia coli* and staphylococcus. The extract was obtained through soxhlet extraction with Acetone, dichloromethane and n-haxane, while the antibacterial activity conducted using disc diffusion method. The crude extract has shown a remarkable inhibition against *S. aureus* (21.00 ±0.01mm) compared to *E. coli* (14±0.02 mm). However, N-hexane had no antibacterial activity against the tested organisms. In another study, the *in vitro* antibacterial activity of Korean Endemic Citrus Species tested against *propionibacterium acnes* and *staphylococcus* epidermidis revealed that nine (9) out of fourteen citrus species citrus oils exhibited antibacterial activity against *P. Acne,* but not against *S. Epidermidis* [45].

CONCLUSION

This review confirmed the antimicrobial activity of essential oils from the peels of different citrus fruits against wide range of bacteria and fungi. The antimicrobial activity was found to be due to the phytochemical compounds present in the peels oil. However, further studies are needed for prospective applications in nutraceutical, microencapsulation and nanotechnology industries. For all the available extraction methods for citrus peel EOs, HD was the most widely used. This may be because of its low cost, efficiency and higher yield. However, there are some other techniques such as ultrasound-assisted extraction, supercritical and subcritical fluid extraction and enzymes-assisted extraction that have not been widely used and their literatures appear to be very scarce.

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